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(54) Title: CRMP-5 NUCLEIC ACID, POLYPEPTIDE AND USES THEREOF

(57) Abstract: The present invention provides for CRMP-5 nucleic acids and polypeptides. CRMP-5 polypeptides have utility in detecting anti-CRMP-5 autoantibodies in an individual exhibiting paraneoplastic neurological manifestations. An association is reported herein between anti-CRMP-5 autoantibodies and neoplasms. Additionally, antibodies with specific binding affinity for CRMP-5 are provided by the present invention. Monoclonal antibodies have utility in detecting the presence of CRMP-5 polypeptides in individuals, based on an association reported herein between CRMP-5 polypeptides and neoplasms.

# CRMP-5 NUCLEIC ACID, POLYPEPTIDE AND USES THEREOF

## FEDERALLY SPONSORED RESEARCH

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## TECHNICAL FIELD

This invention relates generally to paraneoplastic neurological syndromes and cancer, and more particularly, to the involvement of CRMP-5 polypeptides in paraneoplastic syndromes and cancer. The present invention provides for CRMP-5 nucleic acids and polypeptides and uses thereof.

## BACKGROUND

Neurological manifestations of paraneoplastic autoimmunity reflect an anti-tumor immune response against neuron and muscle autoantigens expressed in a cancer. Neoplasms expressing these organ-restricted proteins as immunogens include thymomas and carcinomas of the lung, ovary, breast and testis. It is clear that both host and tumor factors are determinants of paraneoplastic immune responses, but molecular determinants of immunogenicity in individual tumors are unknown.

A number of onconeural antigens have been defined in the nucleus, cytoplasm and plasma membrane of neurons and muscle by their specific reactivity with autoantibodies in patients' sera. Antibodies reactive with cation channels and neurotransmitter receptors residing in synaptic plasma membranes have the potential to interfere with neural transmission in the peripheral nervous system and the central nervous system. Autoantibodies directed at intracellular antigens are unlikely to be either neuropathogenic or inhibitory to tumor growth, but those of the IgG class reflect helper T cell activation. IgG autoantibodies of neuronal nuclear and certain cytoplasmic specificities are usually accompanied by inflammatory lesions in the neuraxis and a distant occult neoplasm with

limited metastasis. These autoantibodies lack demonstrable pathogenicity, but are surrogate markers of T lymphocyte activation, and predict specific cancers.

## SUMMARY

The present invention describes a novel IgG and its antigen. The novel IgG is an anti-neuronal autoantibody marker of limited small-cell lung carcinoma and thymoma in individuals with unusual multifocal neurological disorders. The antigen is a novel isoform of the neuronal collapsin response-mediator protein (CRMP) family and was designated CRMP-5. CRMP-5 is highly expressed in neurons throughout the adult nervous system and is expressed in small-cell lung carcinomas and neuroblastoma.

In one aspect, the present invention provides for an isolated nucleic acid selected from the following: (a) an isolated nucleic acid having the nucleotide sequence of SEQ ID NO:1; (b) a fragment of the nucleic acid of (a), wherein the fragment is: (i) a fragment consisting of nucleotide 1 through at least nucleotide 544 of SEQ ID NO:1; (ii) a fragment of at least 70 nucleotides in length from nucleotide 544 through nucleotide 1695 of SEQ ID NO:1; or (iii) a fragment of at least 70 nucleotides in length comprising nucleotide 544 of SEQ ID NO:1 within the at least 70 nucleotide fragment; (c) a nucleic acid that is at least 92% identical to (a) or (b); and (d) a nucleic acid complementary to (a), (b) or (c). The above-described nucleic acid may encode a polypeptide having the amino acid sequence shown in SEQ ID NO:2, which is preferably a CRMP-5 polypeptide.

Also included in the invention are vectors containing an above-described nucleic acid, and host cells containing those vectors. Additionally, a vector may include regulatory elements that are necessary for expression and that are operably linked to the nucleic acid. The present invention further provides host cells containing those expression vectors. Representative host cells include bacterial, yeast, insect and animal cells. Additionally provided by the invention is a method of producing a CRMP-5 polypeptide, comprising the steps of: (a) culturing host cells containing the above-described expression vector under conditions permissive for expression of the nucleic acid; and (b) recovering polypeptides resulting from the expression of the nucleic acid.

In another aspect, the invention provides for a method of detecting the presence or absence of an anti-CRMP-5 autoantibody in an individual's biological sample. This method comprises the steps of: (a) contacting the biological sample with a CRMP-5 polypeptide or fragment thereof; and (b) detecting the presence or absence of binding of the CRMP-5 polypeptide to the anti-CRMP-5 autoantibody in the biological sample.

Typically, the presence of the anti-CRMP-5 autoantibody in the biological sample is associated with paraneoplastic autoimmunity in the individual, which is, in turn, typically associated with a neoplasm, such as small-cell lung carcinoma, neuroblastoma and thymoma, in the individual. Representative biological samples include blood, serum and cerebrospinal fluid.

In yet another aspect of the invention, there is provided an antibody (*i.e.*, polyclonal or monoclonal) having specific binding affinity for a CRMP-5 polypeptide. A monoclonal antibody having specific binding affinity for a CRMP-5 polypeptide may be produced by a hybridoma cell line such as CR1 or CR3.

Another aspect of the invention is a method of detecting the presence or absence of a CRMP-5 polypeptide in a biological sample from an individual. This method comprises the steps of: (a) contacting the biological sample with an antibody having specific binding affinity for a CRMP-5 polypeptide; and (b) detecting binding of the antibody to the biological sample. Binding is indicative of the presence of the CRMP-5 polypeptide in the biological sample, and the presence of the CRMP-5 polypeptide in the biological sample is indicative of a neoplasm. Generally, the neoplasm is small-cell lung carcinoma, thymoma or neuroblastoma. Representative examples of biological samples are blood, serum, cerebrospinal fluid, pleural fluid, ascites, saliva, sputum, urine, stool or solid tissues.

The present invention also provides for a kit containing a CRMP-5 polypeptide. The kit may further comprise a monoclonal antibody having specific binding affinity for a CRMP-5 polypeptide.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those

described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In addition, the materials, methods and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the drawings and detailed description, and from the claims.

## DESCRIPTION OF DRAWINGS

*Figure 1* is the cDNA and predicted polypeptide sequence of human CRMP-5. The underlined amino acids represent the polypeptide fragments generated by proteolytic cleavage of the polypeptide isolated from human brain.

*Figure 2* is a restriction map of a CRMP-5 nucleic acid. A, *Asp*I; B, *Bam*HI; H, *Hind*III; N, *Nco*I; P, *Pvu*II; R, *Rca*I; S, *Sac*I.

Like reference symbols in the various drawings indicate like elements.

## DETAILED DESCRIPTION

Since 1993, the Clinical Neuroimmunology Laboratory of the Mayo Clinic has performed prospective serological tests on approximately 56,000 individuals. Most presented with abnormal neurological symptoms suspected to be of paraneoplastic origin. Immunofluorescence screening followed by Western blot analyses identified 106 individuals with a novel IgG autoantibody that bound to a 62-kD neuronal cytoplasmic polypeptide. Clinical history, physical findings and laboratory data were available for 102 of those 106 individuals.

The human 62-kD antigen was immunochemically identified and purified, and the oncological and neurological correlations between the novel autoantibody and the novel

antigen are reported herein. From partial amino acid sequence data from the purified antigen, a cDNA encoding the antigen was cloned and its tumor expression and antigenicity demonstrated. The previously undescribed onconeural antigen is related to the collapsin-response-mediator protein (CRMP) family involved in axon guidance in the nervous system and was designated CRMP-5. This invention defines CRMP-5 as a novel onconeuronal protein that elicits spontaneous immune responses in patients with small-cell lung carcinoma and thymoma. The invention also defines IgG autoantibodies specific for CRMP-5 and provides examples of their potential clinical applications.

The novel CRMP-5 onconeuronal antigen was assigned to the collapsin response-mediator protein (CRMP) family (averaging 50% amino acid identity with the other CRMP family members, Table 1) rather than a dihydropyriminidase (DHPase; 57% identity with CRMP-5, Table 1), based on the following: CRMP-5 lacks one of four invariant histidines critical for DHPase enzymatic activity (*i.e.*, CRMP-5 contains conserved histidine (H) residues at positions 68, 70 and 191 of SEQ ID NO:2 and an asparagine (N) instead of an H at position 247), and expression of CRMP-5 is restricted to the nervous system.

Table 1

Amino Acid Identities (%) Among Human CRMP* Proteins and DHPase†

| | CRMP-1 | CRMP-2 | CRMP-3 | CRMP-4 | CRMP-5 | DHPase |
|---|---|---|---|---|---|---|
| CRMP-1 | / | 70 | 67 | 69 | 50 | 58 |
| CRMP-2 | 70 | / | 74 | 74 | 51 | 58 |
| CRMP-3 | 67 | 74 | / | 68 | 49 | 58 |
| CRMP-4 | 69 | 74 | 68 | / | 50 | 58 |
| CRMP-5 | 50 | 51 | 49 | 50 | / | 57 |
| DHPase | 58 | 58 | 58 | 58 | 57 | / |

*GenBank Accession numbers for CRMP-1, 2, 3, 4, 5: Q14194, Q16555, Q14531, Q14195 and AF157634, respectively.

†GenBank Accession number for DHPase: Q14117.

*Human CRMP-5 Nucleic Acids*

In one aspect, the invention features an isolated nucleic acid encoding a human CRMP-5 polypeptide, and the complement thereof. As used herein, nucleic acid refers to RNA or DNA. The invention also features CRMP-5 polypeptides. The term "CRMP-5" refers to a novel polypeptide of the neuronal collapsin response-mediator protein (CRMP) family. CRMP-5 is expressed in neurons of the adult central and peripheral nervous system, in small-cell lung carcinomas and neuroblastomas.

As used herein with respect to nucleic acids, "isolated" refers to (i) a nucleic acid sequence encoding part or all of the human CRMP-5 polypeptide, but free of coding sequences that normally flank one or both sides of the nucleic acid sequences encoding CRMP-5 in the human genome; or (ii) a nucleic acid incorporated into a vector or into the genomic DNA of an organism such that the resulting molecule is not identical to any naturally-occurring vector or genomic DNA.

With respect to polypetides, "isolated" refers to a polypeptide that constitutes the major component in a mixture of components, *e.g.*, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, or 95% or more by weight. Isolated polypeptides are typically obtained by purification from an organism that makes the polypeptide, although chemical synthesis is also feasible. Methods of polypeptide purification include, for example, chromatography or immunoaffinity techniques.

An example of a nucleotide sequence encoding a human CRMP-5 polypeptide is shown in Figure 1 (SEQ ID NO:1), which is a 1695 bp cDNA having an open reading frame of 1695 nucleotides. The predicted amino acid sequence of a human CRMP-5 is shown in SEQ ID NO:2, and is 564 amino acids in length.

In another aspect, the invention includes fragments of the human CRMP-5 nucleic acid and polypeptide. As used herein, fragments of the invention refer to nucleic acids or polypeptides corresponding to less than the entire CRMP-5 sequence. Nucleic acid fragments may include those fragments starting at nucleotide 1 and continuing through at least nucleotide 544 of SEQ ID NO:1; fragments of at least 70 nucleotides in length between nucleotide 544 and nucleotide 1695 (inclusive) of SEQ ID NO:1; or those fragments of at least 70 nucleotides in length that include nucleotide 544 of SEQ ID NO:1 within the 70 nucleotide or greater fragment. Fragments provided by the invention include, for example, fragments from nucleotides 551-650, 1001-1100 or 1401-1500 of

SEQ ID NO:1. The invention further provides novel fragments that are less than 70 nucleotides and that have utility as hybridization probes or PCR primers (*e.g.*, nucleotides 738-774, 1115-1133 or 1543-1557 of SEQ ID NO:1). Additional fragments of the invention include, for example, nucleotides 26-775, 775-929, 1147-1652 or 479-1408 of SEQ ID NO:1. Such fragments may, for example, encode a CRMP-5 antigenic polypeptide fragment.

Figure 2 shows the relative position of various restriction enzyme sites within the human CRMP-5 nucleic acid sequence that, by way of example, define positions, which, in various combinations, can be used to generate useful nucleic acid fragments. Given the nucleotide sequence of a human CRMP-5 polypeptide, virtually any nucleic acid fragment can be generated by known means (*e.g.*, restriction enzyme digestion, the polymerase chain reaction) and, if so desired, expressed to produce the corresponding polypeptide fragment. Alternatively, the human CRMP-5 polypeptide can be cleaved (*e.g.*, proteolytically) to directly generate polypeptide fragments. Representative examples of CRMP-5 fragments generated by proteolytic cleavage are shown underlined in Figure 1.

A human CRMP-5 nucleic acid or nucleic acid fragment may have a sequence that deviates from that shown in SEQ ID NO:1 or fragment thereof. For example, a nucleic acid sequence can have at least 92 percent (%) sequence identity to the nucleotide sequence of SEQ ID NO:1. In some embodiments, the nucleic acid sequence can have at least 95% sequence identity or at least 99% sequence identity to SEQ ID NO:1.

Percent sequence identity is calculated by determining the number of matched positions in aligned nucleic acid or polypeptide sequences, dividing the number of matched positions by the total number of aligned nucleotides or amino acids, respectively, and multiplying by 100. A matched position refers to a position in which identical nucleotides or amino acids occur at the same position in aligned sequences. The total number of aligned nucleotides or amino acids refers to the minimum number of CRMP-5 nucleotides or amino acids, as disclosed in SEQ ID NO:1 or 2, respectively, that are necessary to align the second sequence, and does not include alignment (*e.g.*, forced alignment) with non-CRMP-5 sequences, such as those fused to CRMP-5. The total number of aligned nucleotides or amino acids may correspond to the entire CRMP-5 sequence (*i.e.*, 1695 nucleotides or 560

amino acids) or may correspond to fragments of the full-length CRMP-5 sequence as defined herein. Sequences can be aligned using the Sequence Analysis Software Package of the Genetics Computer Group (University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Sequence (*i.e.*, pairwise) analysis of human CRMP-5 was performed with GCG Gap using the algorithm of Needleman & Wunsch (*J. Mol. Biol.*, 48:443-53 (1970)) and default parameters (a Gap Weight of 50 and a Length Weight of 3).

A nucleic acid encoding a human CRMP-5 polypeptide may be obtained from, for example, a cDNA library made from a human small-cell lung carcinoma cell line, or can be obtained by other means, including, but not limited to, the polymerase chain reaction (PCR). PCR refers to a procedure or technique in which target nucleic acids are amplified. PCR can be used to amplify specific sequences from DNA as well as RNA, including sequences from total genomic DNA or total cellular RNA. Various PCR methods are described, for example, in *PCR Primer: A Laboratory Manual,* Dieffenbach & Dveksler, Eds., Cold Spring Harbor Laboratory Press, 1995. Generally, sequence information from the ends of the region of interest or beyond is employed to design oligonucleotide primers that are identical or similar in sequence to opposite strands of the template to be amplified. Representative oligonucleotide primers for CRMP family members (including DHPase) are shown in Table 2 and SEQ ID NOS:5-28

Human CRMP-5 nucleic acids can be detected by, for example, a variety of hybridization techniques. Hybridization typically involves Southern or Northern blotting (see, for example, sections 9.37-9.52 of Sambrook et al., 1989, *"Molecular Cloning, A Laboratory Manual"*, 2nd Edition, Cold Spring Harbor Press, Plainview; NY). Oligonucleotides should hybridize under high stringency conditions to a human CRMP-5 nucleic acid (*e.g.*, DNA or RNA), or the complement thereof. High stringency conditions typically include the use of low ionic strength and high temperature washes, for example 0.015 M NaCl/0.0015 M sodium citrate (0.1X SSC), 0.1% sodium dodecyl sulfate (SDS) at 65°C. Denaturing agents, such as formamide, can additionally be employed during high stringency hybridization (*e.g.*, 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C).

Table 2

| Name | SEQ ID NO: | Primers | Size |
|---|---|---|---|
| DHPASE, dihydropyrimidinase (Hydroantoinase, DHP) (GenBank Accession No. D78011) | 5<br>6<br>7<br>8 | DHP1: tgg tca acg atg act tct cgg<br>DHP2: tca ggg gtg ggc ctg ttt cct<br>DHP3: cag acc tgg agc tgt acg aag<br>DHP4: tcc cat att acg gac atc cga | 1519bp<br><br>571bp |
| CRMP-1, collapsin response mediator protein-1 (DRP-1, dihydropyrimidinase related protein-1) (GenBank Accession No. D78012) | 9<br>10<br>11<br>12 | CRMP1-1: atg tcg tac cag ggc aag aag<br>CRMP1-2: acc gag atg gac atg att ccc<br>CRMP1-3: cat cac aag ctg gta cga tgg<br>CRMP1-4: ttc ctg att ttg acg cgc tgg | 1715bp<br><br>1037bp |
| CRMP-2 (DRP-2) (GenBank Accession No. D78013) | 13<br>14<br>15<br>16 | CRMP2-1: ccc agg aga gag atg tct tat c<br>CRMP2-2: cta gcc cag gct ggt gat gtt<br>CRMP2-3: aga tcg ctt cca gct aac gga<br>CRMP2-4: tgg ttt taa cgc tgt cgg ggt c | 1731bp<br><br>744bp<br> |
| CRMP-3 (DRP-4, ULIP-4, Cytosolic phosphoprotein) (GenBank Accession No. AB006713) | 17<br>18<br>19<br>20 | CRMP4-1: gga tgt cct tcc agg gca aga<br>CRMP4-2: cta gga gag aga ggt gat gtt g<br>CRMP4-3: gac gac cag tcc ttt tac gat<br>CRMP4-4: cgg atg atg ctg aag atc tcg | 1720bp<br><br>487bp |
| CRMP-4 (DRP-3, or unc-33 like 'Protein phosphoprotein) (GenBank Accession No. D78014) | 21<br>22<br>23<br>24 | CRMP3-1:cca tgt cct acc aag gca aga<br>CRMP3-2: ctc tct ttg agg aag gct tgc<br>CRMP3-3: tgg acg aaa acc agt tcg tgg<br>CRMP3-4: ggt caa aca cag gcc cat cgt | 1735bp<br><br>392bp |
| CRMP-5 (GenBank Accession No. AF157634) | 25<br>26<br><br>27<br>28 | Forward: atg ctt gcc aac tca gcc agc gtg<br>Reverse: gcc ttt acc aaa tgc cac tcg acc<br>Degenerate primers:<br>TK2( F ): gtn ath ccn ggn ggn ath gay ac<br>TK3( R ): ggn gtn ckr tcn acn ccn ckn ac | 1699bp<br><br><br>approx. 1100bp |

DHPase, CRMP1-4: primer 1 and 2 were used for full length PCR; primer 3 and 4 were used for nest PCR.

CRMP-5: Forward and Reverse primers were used for cloning the full length cDNA from both human brain and SCC-9 cDNA pools; TK2 (F), and TK3 (R ) were degenerate primers corresponding to peptides VIPGGI and VRGVDRTP for initial CRMP-5 cDNA cloning.

I.U.P.A.C. codes for bases: r=A, G; m=A, C; w=A, T; y=T, C; k=T, G; s=G, C; b=T, G, C; v=A, G, C; h=A, T, C; d=A, T, G; n=A, T, G, C.

*Human CRMP-5 Nucleic Acid Constructs*

The present invention further includes vectors containing the human CRMP-5 nucleic acid of SEQ ID NO:1 (or the complement thereof), CRMP-5 nucleic acid fragments (or the complements thereof) and those nucleic acids having at least 92% sequence identity to SEQ ID NO:1 or fragments generated therefrom (or the complements thereof).

Cloning vectors suitable for use in the present invention are commercially available and used routinely by those of ordinary skill. Vectors of the invention may additionally comprise elements necessary for expression operably linked to a human CRMP-5 nucleic acid sequence. "Elements necessary for expression" include promoter sequences, and additionally may include regulatory elements, such as enhancer sequences, response elements or inducible elements that modulate expression of the human CRMP-5 nucleic acid sequence. As used herein, "operably linked" refers to positioning of a promoter and/or other regulatory element(s) in a construct relative to the human CRMP-5 nucleic acid sequences in such a way as to direct or regulate expression of the CRMP-5 nucleic acid. Such constructs are commercially available (*e.g.*, expression vectors) and/or produced by recombinant DNA technology methods routine in the art. The choice of expression systems depends upon several factors, including, but not limited to, replication efficiency, selectability, inducibility, targeting, the level of expression desired, ease of recovery and the ability of the host to perform post-translational modifications.

As used herein, the term "host" or "host cell" is meant to include not only prokaryotes, such as *E. coli*, but also eukaryotes, such as yeast, insect, plant and animal cells. Animal cells include, for example, COS cells and HeLa cells. A host cell can be transformed or transfected with a DNA molecule (*e.g.*, a vector) using any of the techniques commonly known to those of ordinary skill in this art, such as calcium phosphate or lithium acetate precipitation, electroporation, lipofection and particle

bombardment. Host cells containing a vector of the present invention may be used for purposes such as propagating the vector, producing human CRMP-5 nucleic acid (*e.g.*, DNA, RNA, antisense RNA) or expressing the human CRMP-5 polypeptide or fragments thereof.

In another aspect of the invention, methods of producing CRMP-5 polypeptides are provided. Methods of producing CRMP-5 polypeptides include, but are not limited to, culturing host cells containing a CRMP-5 expression vector under conditions permissive for expression of CRMP-5, and recovering the CRMP-5 polypeptides. Methods of culturing bacteria and recovering expressed polypeptides are well known to those of ordinary skill in this art.

Additionally, nucleic acids of the present invention may be detected by methods such as Southern or Northern blot analysis (*i.e.,* hybridization), PCR or *in situ* hybridization analysis. CRMP-5 proteins are typically detected by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis followed by Coomassie Blue-staining or Western blot analysis, using antibodies (monoclonal or polyclonal) that have specific binding affinity for a human CRMP-5 polypeptide.

*CRMP-5 Antigen and Anti-CRMP-5 Antibodies*

The present invention provides for methods of using CRMP-5 polypeptides to detect anti-CRMP-5 autoantibodies in an individual. The individual is typically displaying abnormal neurological symptoms of unknown origin. The method of the invention is based on an association between the abnormal neurological symptoms, the presence of the anti-CRMP-5 autoantibody and the presence of neoplasms (*e.g.*, neuroendocrine neoplasms, such as small-cell lung carcinoma, thymoma and neuroblastoma). Representative abnormal neurological symptoms associated with paraneoplastic autoimmunity are shown in Table 4.

The polypeptides and polypeptide fragments used for detection of anti-CRMP-5 autoantibodies in the methods of the present invention are specifically reactive with anti-CRMP-5 autoantibodies. Polypeptides generally correspond to at least one epitopic site that is characteristic of a CRMP-5 protein. Epitopes of the CRMP-5 polypeptide that are pertinent to T-cell activation and suppression are also provided by the invention.

Computer algorithms are available for predicting binding epitopes, *e.g.*, MHC-I and MHC-II binding epitopes. See, for example, http://bimas.dcrt.nih.gov:80/molbio/hla bind/ (Parker et al., *J. Immunol.*, 152:163 (1994); Southwood et al., *J. Immunol.*, 160:3363 (1998)). The term "characteristic" in this context means that the epitopic site allows immunologic detection of anti-CRMP-5 antibody or antigenic CRMP-5 polypeptides in sera with reasonable assurance. Usually, it is desirable that the epitopic site be antigenically distinct from other members of the CRMP family.

The CMRP-5 polypeptides may be obtained from cells (*e.g.*, transfected host cells) expressing a CRMP-5 nucleic acid, or the polypeptides may be synthetic. A DNA molecule encoding a CRMP-5 polypeptide of fragment thereof may itself be natural or synthetic, with natural genes obtainable from human tissues by conventional techniques.

To be useful in the detection methods of the present invention, the CRMP-5 polypeptides are obtained in a substantially pure form. The polypeptides may be purified by routine protein purification methods, including affinity chromatography (*e.g.*, as described herein), or immunosorbant affinity column.

CRMP-5 polypeptides of the present invention may be used with or without modification for the detection of CRMP-5. Frequently, polypeptides are labeled by either covalently or non-covalently combining the polypeptide with a second substance that provides for detectable signal. A wide variety of labels and conjugation techniques are known in the art and are reported extensively in both the scientific and patent literature. Some of the labels include radioisotopes, enzymes, substrates, cofactors, inhibitors, fluorescers, chemiluminescers, magnetic particles and the like.

CRMP-5 polypeptides prepared as described above can be used in various immunological techniques for detecting anti-CRMP-5 autoantibodies in serum samples, such as from blood and cerebrospinal fluid. Depending on the nature of the sample, both immunoassays and immunocytochemical staining techniques may be used. Enzyme-linked immunosorbent assays (ELISA), Western blot and radioimmunoassays are routine methods in the art and may be used to detect the presence of anti-CRMP-5 autoantibodies in sera.

Further provided by the present invention are kits containing CRMP-5 polypeptides. The kit may further include a second substance that provides for detectable

signal. A kit typically also includes directions for using the CRMP-5 polypeptide and/or practicing the method (*i.e.*, detecting anti-CRMP-5 autoantibody).

The present invention also provides a method of detecting CRMP-5 polypeptides in a biological sample from an individual. The method describes an association between the presence of CRMP-5 and neoplasms (*e.g.*, small-cell lung carcinomas and neuroblastomas) in the individuals screened. This test is most widely applicable to those individuals who do not present with abnormal neurological symptoms, but are suspected to have a new or recurrent neoplasm, *e.g.*, small-cell lung carcinoma. Detection of a protein is typically performed using an antibody, and the present invention also provides for an antibody, preferably a monoclonal antibody with specific binding affinity for CRMP-5 polypeptides.

Once a sufficient quantity of CRMP-5 polypeptides has been obtained, monoclonal or polyclonal antibodies having specific binding affinity for CRMP-5 may be produced by techniques well known to those of ordinary skill in the art to which this invention pertains. As used herein, antibodies having "specific binding affinity" for CRMP-5 are defined as antibodies that bind CRMP-5 but that do not bind, for example, either CRMP-2 or CRMP-3. As used herein, "antibody" refers to whole antibodies of any class, *i.e.*, IgG, IgA, IgM or any other known class, and includes portions or fragments of whole antibodies (*e.g.*, Fab or $(Fab)_2$ fragments) having the desired affinity, an engineered single chain Fv molecule, or a chimeric molecule, *e.g.*, an antibody that contains the binding specificity of one antibody (*e.g.*, of murine origin) and the remaining portions of another antibody (*e.g.*, of human origin). Hybridomas that produce monoclonal antibodies having specific binding affinity for CRMP-5 have been deposited with ATCC (10801 University Blvd., Manassas, VA 20110-2209) and were assigned the following Accession number(s):

| Hybridoma | Accession No. | Date Deposited |
|---|---|---|
| CR-1 | PTA-2164 | June 28, 2000 |
| CR-3 | PTA-2165 | June 28, 2000 |

Anti-CRMP-5 antibodies of the present invention may be used with or without modification for the detection of CRMP-5. Frequently, antibodies are labeled either directly or indirectly, and a wide variety of labels, including radioisotopes, enzymes, substrates, cofactors, inhibitors, fluorescers, chemiluminescers and magnetic particles, and conjugation techniques are known and are reported extensively in both the scientific and patent literature.

Antibodies prepared as described above can be used in various immunological techniques for detecting CRMP-5 polypeptides in a biological sample. A "biological sample", as used herein, is generally a sample from an individual. Non-limiting examples of biological samples include blood, serum, or cerebrospinal fluid, pleural fluid, ascites, saliva, sputum, urine or stool. Additionally, solid tissues, for example, lymph node specimens, may be used, *e.g.*, for intraoperative diagnosis. The use of antibodies in protein binding assays is well established. Depending on the nature of the sample, immunoassays (*e.g.*, radioimmunoassays) and/or immunohistochemical/immunocytochemical staining techniques may be used. Liquid phase immunoassays or Western blot analysis can also be used to detect CRMP-5 in a biological sample. Additionally, enzyme-linked immunosorbent assays (ELISA) are routinely practiced in the art, and may be used for detecting the presence of CRMP-5 in a biological sample.

Numerous competitive and noncompetitive protein-binding assays have been described in the scientific and patent literature, and a large number of such assays are commercially available. An example of one such competitive assay for detecting the presence of, for instance, the CRMP-5 polypeptide in a biological sample such as blood, comprises: contacting a CRMP-5 polypeptide (either labeled or unlabeled) with an anti-CRMP-5 antibody (either labeled or unlabeled) and the biological sample. The CRMP-5 polypeptide may be, for example, attached to a solid surface. Using known amounts of CRMP-5 polypeptide and labeled anti-CRMP-5 antibody to generate a standard binding curve, the relative amount of CRMP-5 polypeptide in a biological sample can be determined.

Further provided by the invention is a kit containing antibodies having binding affinity for CRMP-5 polypeptides or fragments thereof. The kit may also include CMRP-

5 polypeptides or fragments thereof to be used as binding controls or to generate a standardized quantitative curve. The kit may further include a second substance that provides for detectable label. A kit typically includes directions for using the anti-CRMP-5 antibody and/or practicing the method (*i.e.*, detecting CRMP-5 polypeptides).

Also provided by this invention is an antibody having specific binding affinity for CRMP-5 conjugated to a detectable marker. Suitable detectable markers include, but are not limited to, enzymes, radioisotopes, dyes and biotin. This invention further provides an antibody having specific binding affinity for CRMP-5 conjugated to an imaging agent. Suitable imaging agents include, but are not limited to, radioisotopes, such as $^{32}P$, $^{99}Tc$, $^{111}In$ and $^{131}I$.

Also provided by this invention are pharmaceutical compositions comprising CRMP-5 polypeptide, alone or conjugated to a detectable marker, and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as phosphate buffered saline solution, water, emulsions (*e.g.*, oil/water) or various types of wetting agents.

Further provided by the invention are methods of immunomodulating a neurological disorder in an individual, resulting from CRMP-5-specific cellular immune mechanisms. The method comprises administering an effective amount of a pharmaceutical composition of a CRMP-5 polypeptide or a nucleic acid encoding the CRMP-5 polypeptide to the individual. An effective amount is an amount or CRMP-5 polypeptide that deviates the individual's CRMP-5-mediated immune response, thereby modulating a neurological disorder in the individual. As used herein, "administering" means a method of administering to the patient. Such methods are well known to those skilled in the art and include, but are not limited to, administration orally, intravenously, or parenterally.

Also provided by this invention is a method of imaging CRMP-5-expressing neoplastic cells in a patient. The method comprises administering to the patient an effective amount of an antibody having specific binding affinity for CRMP-5, labeled with an imaging agent, for example, $^{32}P$, $^{99}Tc$, $^{111}In$ or $^{131}I$, to bind to a CRMP-5 antigen released from, or accessible in, neoplastic cells shed from the tumor, and detecting any complex so formed. As is well known to those of ordinary skill in the art, a suitable

amount of antibody or composition is any amount that is effective to image the neoplastic cells, for example, about 0.1 mCi to about 50.0 mCi. In addition, an effective amount of the antibody may be an amount from about 0.01 mg to about 100 mg. Suitable methods of administering the imaging agent are as described above. Methods of imaging are dependent upon the agent used and are well known to those of skill in this art.

Further provided by this invention are methods of killing or inhibiting the proliferation of neoplastic cells in a patient. One method comprises administering to the patient, or to isolated antigen presenting cells (APCs) from the patient, an effective amount of a vaccine to stimulate cytotoxic T-cells. The vaccine is a pharmaceutical composition comprising the CRMP-5 polypeptide of the invention, or a nucleic acid encoding the CRMP-5 polypeptide. The CRMP-5 polypeptide may be co-administered to the patient with an immunomodulatory or immunostimulatory molecule, or administered via at least one nucleic acid encoding a CRMP-5 polypeptide and an immunomodulatory/immunostimulatory molecule (*e.g.*, a single nucleic acid may encode a CRMP-5-immunomodulatory or CRMP-5-immunostimulatory fusion protein). For the purposes of this invention, suitable immunomodulatory or immunostimulatory molecules include, for example, cytokines (*e.g.*, GM-CSF, IL-12, IL-10) or unmethylated CpG sequences. An effective amount is an amount that effectively modulates or stimulates the patient's immune response such that the neoplastic cells are killed or their proliferation inhibited.

Also provided is a method of enumerating or isolating CRMP-5-specific T-lymphocytes in an individual. This method may be used, for example, to monitor an individual's immune response or for immunotherapy using CRMP-5 antigen-specific cytotoxic T-cells. The method comprises contacting a patient-derived biological sample containing lymphocytes with tetrameric soluble class I major histocompatibility complex (MHC) bearing identical antigenic CRMP-5 polypeptide fragments. Linker molecules, such as avidin and biotin, are used to produce the CRMP-5-MHC tetrameric complex and can subsequently be labeled with an indicator molecule, such that those T-cells that recognize the CRMP-5-MHC tetrameric complex are enumerated or isolated (*e.g.*, using FACS analysis). See, for example, Schwartz, RS, *New England J. Med.*, 339:1076-8, 1998, and references therein.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

## EXAMPLES

### Example 1—Protein Purification and Partial Sequencing of the CRMP-5 Antigen

All steps were at 4°C. Buffers contained protease inhibitors (phenylmethylsulfonyl fluoride, 2 mM; Pepstatin A, 0.1 μg/ml; and Aprotinin, 1 KIU/ml). Human cerebral grey matter was homogenized (1 g/10 ml) in 10 mM N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid] (HEPES) buffer (pH 7.4), containing 1 mM $MgCl_2$, 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). The Mayo Clinic Institutional Review Board approved acquisition of surgical waste tissues (IRB #69-91) and review of patients' histories for this study (IRB #863-98).

The supernate from ultracentrifugation (100,000 xg; 1 h) was sequentially precipitated by 30% and 40% ammonium sulfate. Polypeptides in the second precipitate were dissolved in phosphate-buffered saline (pH 7.4), dialyzed against 10 mM HEPES (pH 7.4) containing 500 mM NaCl, and applied sequentially to two affinity resins (Affi-gel 10, Bio-Rad Laboratories, Hercules, CA) coupled with IgGs (10 mg/ml) purified by protein G-agarose adsorption from serum of a healthy subject or a reactive individual. Polypeptides that bound to the second resin were eluted in 6 M urea containing 2% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) (CHAPS), concentrated by centrifugation dialysis using a Centricon-10 membrane (Millipore Corporation, Bedford, MA), and analyzed by gel electrophoresis.

Protein bands visualized by Coomassie blue staining were excised and digested with lysyl-endopeptidase (Wako Chemicals USA, Richmond, VA). Peptide products were analyzed by high performance liquid chromatography (PE Biosystems, Foster City, CA) and amino acid sequences were determined (Procise cLC Protein Sequencing Systems and ABI Model 610 A data analysis software, PE Biosystems).

**Example 2—cDNA Cloning of CRMP-5 and Expression of Recombinant CRMP-5**

RNA extracted from the small-cell lung carcinoma line SCC-9 was reverse transcribed to provide first-strand complementary DNA (cDNA). Degenerate oligonucleotide primers encoding two peptide sequences obtained from the brain antigenic polypeptide (VIPGGI (SEQ ID NO:3) and VRGVDRTP (SEQ ID NO:4)) were used to amplify DNA encoding a CRMP-5 antigen by polymerase chain reaction (PCR). A 1.5 Kb cDNA product was ligated into a TA-cloning vector (Invitrogen, Carlsbad, CA), and used to transform competent *E. coli* cells (INVαF'; Invitrogen). DNA sequences of 23 selected clones were compared to sequences in the GenBank database. Three novel clones contained identical DNA segments that precisely encoded the polypeptide sequences obtained from the brain CRMP-5 antigen. The complete 3' end of the CRMP-5 cDNA was obtained by PCR employing rapid amplification of cDNA ends ("RACE"; Clontech Laboratories, Inc., Palo Alto, CA). Specific primers (Table 2; SEQ ID NOS:25 and 26) were subsequently used to amplify full-length cDNAs from a human brain library and from the small-cell lung carcinoma line, SCC-9 (libraries prepared by Stratagene, La Jolla, CA. A full-length CRMP-2 cDNA (GenBank accession Number D78013) was also cloned from the brain library using CRMP-2-specific primers (Table 2; SEQ ID NO:13-16). Each cDNA was cloned by insertion into a TA-cloning vector.

cDNAs encoding full-length CRMP-5 and CRMP-2 polypeptides and two CRMP-5 fragments (amino acid residues 1-181 or 57-351) were inserted into the pTrcHis expression vector (Invitrogen). Recombinant polypeptide synthesis was induced in transformed *E. coli* (Top10 strain) by adding 1 mM isopropyl-β-thio-galactopyranoside. Coomassie blue staining, Western blot and amino acid analyses confirmed the purity and identity of polypeptides isolated by affinity binding to $Ni^{2+}$ (His-bind system; Novagen, Madison, WI).

**Example 3—Characterization of the Autoantibody with Affinity to CRMP-5**

IgG in 106 individuals' sera yielded a striking neural-restricted immunofluorescent staining pattern in a triple tissue substrate. Standard indirect immunofluorescence conditions revealed homogeneous staining over synapse-rich regions of brain and neural elements in the gut wall. Lack of discrete cellular definition

suggested CRMP-5 antigen diffusion. The pattern of staining was readily distinguished from that of previously documented neuronal autoantibodies. When fixation of the tissue was optimized (by rapid tissue freezing and avoidance of moisture accumulation in thawing sections), staining of the cerebellar cortex resembled the synaptic pattern yielded by the paraneoplastic autoantibody, amphiphysin. In adult rat and mouse tissues, both anti-CRMP-5 and anti-amphiphysin autoantibodies bound to cytoplasmic components of neurons (synapse-rich regions and some somata) and to enteric neural elements. Both antibodies spared Purkinje somata, gut smooth muscle and mucosa, and kidney tissue. Immunoperoxidase staining revealed an abundance of both the novel CRMP-5 antigen and amphiphysin in the neuropil and some neuronal bodies throughout the brain, spinal cord and autonomic ganglia; midbrain synaptic staining was most intense. In dorsal root ganglion, the novel CRMP-5 antigen was more intense in small neurons than in large neurons. Amphiphysin immunoreactivity also was more intense in small neurons than large, by comparison with staining by the paraneoplastic autoantibody ANNA-1 (or "anti-Hu"). Normal IgG did not bind to any elements.

The novel anti-CRMP-5 autoantibody bound to an aqueous brain protein of apparent molecular weight 62-kD under standard Western blot conditions. Initial analysis of Western blots on the same preparation of aqueous brain protein suggested that the anti-CRMP-5 autoantibody bound to a protein having a molecular weight of 66 kD. Binding by the anti-CRMP-5 autoantibody was readily distinguished from immunoreactive bands yielded by ANNA-1, amphiphysin and other previously characterized paraneoplastic IgG autoantibodies. Under non-reducing conditions, the novel anti-CRMP-5 autoantibody bound an apparent tetrameric form (~264 kD) of the CRMP-5 antigen. Additionally, two-dimensional gel electrophoresis and Western blotting data show that native CRMP-5 exists in phosphorylated and non-phosphorylated forms.

**Example 4—Identification of the Human Brain CRMP-5 Antigen**

The aqueous immunoreactive antigen isolated from human brain yielded eight polypeptides after proteolytic cleavage (underlined in Figure 1). All had sequence homology to dihydropyrimidinase (DHPase) and to CRMP family members. A full-length cDNA encoding the brain antigen was cloned from a human adult brain cDNA

pool and assigned GenBank Accession No. AF157634. The predicted polypeptide (Figure 1) contains all eight peptide sequences obtained from cleavage of the purified aqueous brain polypeptide, and has 50% amino acid identity to the previously known four human CRMP family members, and 57% identity to human DHPase (Table 1). Because CRMP-5 lacked one of four invariant histidine residues critical for DHPase activity, the antigen was assigned to a novel CRMP family designated CRMP-5. The predicted size of the CRMP-5 antigen based on the amino acid sequence is 62 kD.

**Example 5—Neurological and Oncological Findings in CRMP-5-Positive Individuals**

Clinical information was available for 102 of the 106 seropositive individuals. Sixty-one were women (aged 46-87) and 41 were men (aged 39-88). The majority of the seropositive individuals had no history, clinical or radiological evidence of a thoracic neoplasm at presentation, and many had no abnormality detected on initial CT scanning of the chest. As of May, 2000, a primary lung carcinoma had been identified in 81 of the 102 individuals (predominantly small-cell type, Table 3). Most of these cancers were remarkably limited in metastasis. The frequent occurrence of thromboembolic phenomena and vasculitic stigmata prior to the detection of lung cancer (Table 3, legend) suggests that systemic cytokine effects may accompany an immune-mediated limitation of cancer spread in CRMP-5-positive individuals. In 43 individuals, the detection of lung cancer required an aggressive, focused and repeated search, as is often the case for ANNA-1-positive patients; 4 were found at autopsy. In 8 individuals who all smoked, other types of neoplasms were found (*e.g.*, carcinomas of colon, skin, breast, prostate and ureter, a B-lymphoma, and an unidentified cerebral mass). The possible existence of an occult primary lung neoplasm was excluded by autopsy in only one individual. All 10 individuals who lacked documentation of a neoplasm had a history of tobacco use, and all but one were known to have had relentless neurological progression; 4 of those died without autopsy.

Thymoma was found in two women (aged 52 and 53) and two men (aged 39 and 40). Two of the four individuals with thymoma had myasthenia gravis, 1 had limbic encephalitis, and 1 had systemic lupus erythematosus. Initial neurological presentations in the other 98 individuals were varied, but the presence of subacute dementia, chorea,

abnormalities of olfaction and taste, and optic neuritis was remarkable in the unusually high frequency in a paraneoplastic context (Table 4). In the majority of individuals, neurological symptoms and signs progressed subacutely to involve multiple levels of the nervous system (Table 4). Mild lymphocytosis, elevated protein and oligoclonal bands were frequently recorded in the spinal fluid, but extrathoracic metastasis was rare.

Table 3

Neoplasms Found in CRMP-5 Seropositive Individuals *

| No. | | Neoplasm |
|---|---|---|
| 81 | | Lung carcinoma † |
| | (67) | Histologically proven |
| | (14) | Imaged |
| 4 | | Thymoma ‡ |
| 8 | | Other neoplasms § |
| 9 | | None ¶ |

*Systemic symptoms and signs were common prior to cancer diagnosis; 26 percent of the 102 individuals had thromboembolic phenomena, vasculitic stigmata, unexplained fever or anemia.

†17% of individuals with primary lung cancer had a co-existing (or past) carcinoma (multiple lung types [3], renal cell [1], prostate [2], breast [2], endometrial [1], colorectal [3], skin, basal cell [3]) or a B-lymphoma (2). The histologically proven lung carcinomas were: small cell (63), large cell (1), squamous cell (2), and adenocarcinoma (1).

‡2 individuals had myasthenia gravis, 1 had limbic encephalitis, and 1 had systemic lupus erythematosus.

§The possibility of occult primary lung cancer was excluded by autopsy in only 1; 6 had 1 or more carcinomas (colon [1], skin, basal cell [2], breast [1], prostate [2], ureteropelvic [1]), 1 had B-lymphoma, 1 had an intracerebral mass.

¶All were smokers and had subacute neurological progression; 4 are known dead without autopsy.

Table 4

Predominant Abnormal Neurological Manifestations in CRMP-5 Seropositive Individuals*

| Level Involved | Total No. | Signs & Symptoms (no. of individuals) |
|---|---|---|
| Cerebral cortex | 42 | dementia (26); personality change (10); seizures (10); depression (9); confusion (8); psychosis (4); aphasia (1) |
| Basal ganglia † | 13 | chorea (10); athetosis (1); Parkinsonism (2) |
| Cerebellum | 26 | ataxia / nystagmus / dysarthria |
| Brainstem | 6 | opsoclonus / myoclonus |
| Cranial nerves | 16 | II (5), VI (1), VII (1), VIII (2); abnormal olfaction/taste (11) |
| Spinal cord | 15 | myelopathy |
| Nerve roots | 2 | polyradiculopathy |
| Somatic nerves / ganglia | 50 | sensory (28); mixed (24); motor (2) |
| Autonomic nerves / ganglia | 33 | mixed (12); gut only (21) |
| Neuromuscular junction | 13 | Lambert-Eaton myasthenic syndrome (10); myasthenia gravis (2); neuromyotonia (1) |

*41 men and 61 women.

†Subacute chorea/athetosis (rarely documented with paraneoplastic autoimmunity) affected 3 men and 8 women. Four of these individuals had coinciding loss of smell and taste. Two men had recent-onset Parkinsonism.

**Example 6—Antigenicity of Recombinant CRMP-5**

IgG in all 102 individuals' sera, but in no control subject's serum, bound to the full-length recombinant CRMP-5 polypeptide in a Western blot. Eleven of 14 representative sera samples bound also to the N-terminal 181 amino acid residues. However, only two of those 14 representative samples bound to a polypeptide

corresponding to amino acid residues 57-351. No individual's IgG bound to the full-length human CRMP-2 polypeptide. These results identify CRMP-5 unambiguously as the neuronal antigen defined serologically herein.

**Example 7—CRMP-5 Antigen Expression in Tumors**

Fifteen small-cell lung carcinoma cell lines were established in the Mayo Clinic's Neuroimmunology Laboratory (SCC-2, 4, 9, 15, 17, 18, 21, 24, 37, 58, 59, 81, 86, 110, 117 and six were obtained from the American Type Culture Collection (ATCC, Manassas, VA; NCI-H69, NCI-H128, NCI-H345, NCI-H146, NCI-H82, NCI-H209). The following human cell lines were obtained from the ATCC and used as controls: IMR-32 (neuroblastoma), TE-671 (rhabdomyosarcoma), Jurkat (T lymphoma), HEK-293 (human embryonic kidney epithelium cells) and HeLa (cervical carcinoma).

RNA transcripts for CRMP-2, CRMP-4 and CRMP-5 were amplified by RT-PCR from a standard small-cell lung carcinoma line (SCC-9). CRMP-1, CRMP-2 CRMP-3, CRMP-4 and CRMP-5 transcripts were amplified from the human brain cDNA library (Table 2; SEQ ID NOS:9-28). The full-length CRMP-5 cDNA sequences obtained from both sources were identical. Western blot analyses revealed CRMP-5 immunoreactivity in the cytosol of all 20 small-cell lung carcinoma lines from individuals (*i.e.*, patients) or from the ATCC. A neuroblastoma (IMR-32) also was immunoreactive, but none of the five non-neuronal cell lines were positive.

**Example 8—Screening for Anti-CRMP-5 Autoantibodies or CRMP-5 Polypeptides**

The CRMP-5 nucleic acid constructs and polypeptides provided by the invention allow for detection of anti-CRMP-5 IgG autoantibodies in an individual's sera. Typically, the presence of the autoantibody correlates with small-cell lung carcinoma or thymoma (Table 3). In the Clinical Neuroimmunology Laboratory's current clinical service activity, the frequency of detection of anti-CRMP-5 autoantibody is approximately 2 per 1,000 sera tested. The CRMP-5 IgG was not encountered in research studies involving large numbers of healthy subjects or individuals with a variety of neurological disorders or neoplasms, with the exception of a single individual not exhibiting any paraneoplastic neurological manifestations out of 58 individuals

previously diagnosed with small-cell lung carcinoma. Additionally, of 14 sera available from 15 Mayo Clinic individuals from whom small-cell lung carcinoma cell lines were derived, only 2 (the donors of SCC-117 and SCC-110) were positive for anti-CRMP-5 autoantibodies. These results indicate that mere expression of CRMP-5 polypeptide in a cancer is not sufficient to stimulate the production of anti-CRMP-5 autoantibodies.

Therefore, the invention also provides for methods to detect the CRMP-5 polypeptide using an anti-CRMP-5 antibody having specific binding affinity for CRMP-5. Since only a small percentage of individuals with small-cell lung carcinomas present with paraneoplastic neurological symptoms, an anti-CRMP-5 monoclonal antibody may be used to screen the general population for early detection of neoplasias (*e.g.*, small-cell lung carcinomas, neuroblastomas and thymomas) based on the presence or absence of CRMP-5 polypeptides. Monoclonal IgGs with specific binding affinity for CRMP-5 polypeptides (*e.g.*, CR1, CR3) were generated by immunizing a rat with native CRMP-5 purified from human brain.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

**WHAT IS CLAIMED IS:**

1. An isolated nucleic acid selected from the group consisting of:

(a) a nucleic acid having the nucleotide sequence of SEQ ID NO:1;

(b) a fragment of the nucleic acid of (a), wherein said fragment is selected from the group consisting of:

(i) a fragment consisting of nucleotide 1 through at least nucleotide 544 of SEQ ID NO:1;

(ii) a fragment of at least 70 nucleotides in length from nucleotide 544 through nucleotide 1695 of SEQ ID NO:1; and

(iii) a fragment of at least 70 nucleotides in length comprising nucleotide 544 of SEQ ID NO:1 within said at least 70 nucleotide fragment;

(c) a nucleic acid that is at least 92% identical to (a) or (b); and

(d) a nucleic acid complementary to (a), (b) or (c).

2. A vector comprising the nucleic acid of claim 1.

3. A host cell comprising the vector of claim 2.

4. The host cell of claim 3, wherein said host cell is selected from the group consisting of bacterial, yeast, insect and animal cells.

5. The vector of claim 2, further comprising regulatory elements necessary for expression operably linked to said nucleic acid.

6. A host cell comprising the vector of claim 5.

7. The host cell of claim 6, wherein said host cell is selected from the group consisting of bacterial, yeast, insect and animal cells.

8. The nucleic acid of claim 1, wherein said nucleic acid encodes a polypeptide having the amino acid sequence shown in SEQ ID NO:2.

9. The nucleic acid of claim 1, wherein said nucleic acid encodes a CRMP-5 polypeptide.

10. A method of producing a CRMP-5 polypeptide, comprising the steps of:

(a) culturing the host cells of claim 6 under conditions permissive for expression of said nucleic acid; and

(b) recovering polypeptides resulting from said expression of said nucleic acid.

11. A method of detecting the presence or absence of an anti-CRMP-5 autoantibody in an individual's biological sample, comprising the steps of:

(a) contacting said biological sample with a CRMP-5 polypeptide or fragment thereof; and

(b) detecting the presence or absence of binding of said CRMP-5 polypeptide to said anti-CRMP-5 autoantibody in said biological sample.

12. The method of claim 11, wherein the presence of said anti-CRMP-5 autoantibody in said biological sample is associated with paraneoplastic autoimmunity in said individual.

13. The method of claim 12, wherein said paraneoplastic autoimmunity is associated with a neoplasm in said individual.

14. The method of claim 11, wherein said biological sample is selected from the group consisting of blood, serum and cerebrospinal fluid.

15. The method of claim 13, wherein said neoplasm is selected from the group consisting of small-cell lung carcinoma, neuroblastoma and thymoma.

16. An antibody having specific binding affinity for a CRMP-5 polypeptide.

17. The antibody of claim 16, wherein said antibody is a monoclonal antibody.

18. The monoclonal antibody of claim 17, wherein said antibody is produced by a hybridoma cell line selected from the group consisting of CR1 and CR3.

19. A method of detecting the presence or absence of a CRMP-5 polypeptide in a biological sample from an individual, comprising the steps of:

(a) contacting said biological sample with the antibody of claim 16; and

(b) detecting binding of said antibody to said biological sample, wherein binding is indicative of the presence of said CRMP-5 polypeptide in said biological sample, wherein the presence of the CRMP-5 polypeptide in said biological sample is indicative of a neoplasm.

20. The method of claim 19, wherein said neoplasm is selected from the group consisting of small-cell lung carcinoma, thymoma and neuroblastoma.

21. The method of claim 19, wherein said biological sample is selected from the group consisting of blood, serum, cerebrospinal fluid, pleural fluid, ascites, saliva, sputum, urine, stool and solid tissues.

22. A kit, comprising a CRMP-5 polypeptide.

23. The kit of claim 22, further comprising a monoclonal antibody having specific binding affinity for a CRMP-5 polypeptide.

```
ATGCTTGCCAACTCAGCCAGCGTGAGGATCCTCATCAAGGGAGGCAAGGTGGTGAACGAT
 M  L  A  N  S  A  S  V  R  I  L  I  K  G  G  K  V  V  N  D
GACTGCACCCACGAGGCTGACGTCTACATCGAGAATGGCATCATCCAGCAGGTGGGCCGC
 D  C  T  H  E  A  D  V  Y  I  E  N  G  I  I  Q  Q  V  G  R
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 G  G  D  F  N  L  Y  E  N  M  R  C  H  G  V  P  L  V  T  I
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 T  L  K  V  R  G  V  D  R  T  P  Y  L  G  D  V  A  V  V  V
CACCCTGGGAAAAAAGAGATGGGAACCCCACTCGCAGACACTCCTACCCGGCCCGTCACC
 H  P  G  K  K  E  M  G  T  P  L  A  D  T  P  T  R  P  V  T
CGGCATGGGGGCATGAGGGACCTTCACGAATCCAGCTTCAGCCTCTCTGGCTCTCAGATC
 R  H  G  G  M  R  D  L  H  E  S  S  F  S  L  S  G  S  Q  I
GATGACCATGTTCCAAAGCGAGCTTCAGCTCGGATCCTCGCTCCTCCCGGAGGCAGGTCG
 D  D  H  V  P  K  R  A  S  A  R  I  L  A  P  P  G  G  R  S
AGTGGCATTTGGTAA      (SEQ ID NO:1)
 S  G  I  W  *       (SEQ ID NO:2)
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FIGURE 1

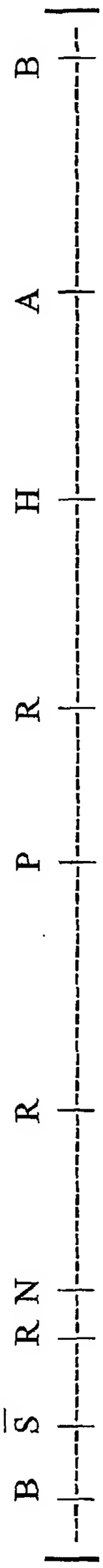


FIGURE 2

SEQUENCE LISTING

<110> Lennon, Vanda A.
Yu, Zhiya
Griesmann, Guy E.
Kryzer, Thomas J.

<120> CRMP-5 NUCLEIC ACID, POLYPEPTIDE AND USES THEREOF

<130> 07039-212001

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gtg gtg aac gat gac tgc acc cac gag gct gac gtc tac atc gag aat        96
Val Val Asn Asp Asp Cys Thr His Glu Ala Asp Val Tyr Ile Glu Asn
            20                  25                  30

ggc atc atc cag cag gtg ggc cgc gag ctc atg atc cct ggc ggg gcc       144
Gly Ile Ile Gln Gln Val Gly Arg Glu Leu Met Ile Pro Gly Gly Ala
        35                  40                  45

aag gtg att gat gcc aca gga aaa ctg gtg atc cct ggt ggc atc gac       192
Lys Val Ile Asp Ala Thr Gly Lys Leu Val Ile Pro Gly Gly Ile Asp
    50                  55                  60

acc agc acc cac ttc cac cag acc ttc atg aat gcc acg tgc gtg gac       240
Thr Ser Thr His Phe His Gln Thr Phe Met Asn Ala Thr Cys Val Asp
65                  70                  75                  80

gac ttc tac cat ggg acc aag gca gca ctc gtc gga ggc acc acc atg       288
Asp Phe Tyr His Gly Thr Lys Ala Ala Leu Val Gly Gly Thr Thr Met
                85                  90                  95

atc atc ggc cat gtc ctg ccc gac aag gag acc tcc ctt gtg gac gct       336
Ile Ile Gly His Val Leu Pro Asp Lys Glu Thr Ser Leu Val Asp Ala
            100                 105                 110
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tat gag aag tgc cga ggt ctg gcc gac ccc aag gtc tgc tgt gat tac      384
Tyr Glu Lys Cys Arg Gly Leu Ala Asp Pro Lys Val Cys Cys Asp Tyr
        115                 120                 125

gcc ctc cac gtg ggg atc acc tgg tgg gca ccc aag gtg aaa gca gaa      432
Ala Leu His Val Gly Ile Thr Trp Trp Ala Pro Lys Val Lys Ala Glu
    130                 135                 140

atg gag aca ctg gtg agg gag aag ggt gtc aac tcg ttc cag atg ttc      480
Met Glu Thr Leu Val Arg Glu Lys Gly Val Asn Ser Phe Gln Met Phe
145                 150                 155                 160

atg acc tac aag gac ctg tac atg ctt cga gac agt gag ctg tac caa      528
Met Thr Tyr Lys Asp Leu Tyr Met Leu Arg Asp Ser Glu Leu Tyr Gln
                165                 170                 175

gtg ttg cac gct tgc aag gac att ggg gca atc gcc cgc gtc cat gct      576
Val Leu His Ala Cys Lys Asp Ile Gly Ala Ile Ala Arg Val His Ala
            180                 185                 190

gaa aat ggg gag ctt gtg gcc gag ggt gct aag gag gca ctg gat ttg      624
Glu Asn Gly Glu Leu Val Ala Glu Gly Ala Lys Glu Ala Leu Asp Leu
        195                 200                 205

ggg atc aca ggc cca gaa gga atc gag atc agc cgt cca gag gag ctg      672
Gly Ile Thr Gly Pro Glu Gly Ile Glu Ile Ser Arg Pro Glu Glu Leu
    210                 215                 220

gaa gct gaa gcc act cat cgt gtt atc acc att gca aac agg act cac      720
Glu Ala Glu Ala Thr His Arg Val Ile Thr Ile Ala Asn Arg Thr His
225                 230                 235                 240

tgt cca atc tac ctg gtc aac gtg tcc agt atc tcg gct ggt gac gtt      768
Cys Pro Ile Tyr Leu Val Asn Val Ser Ser Ile Ser Ala Gly Asp Val
                245                 250                 255

atc gca gct gct aag atg caa ggg aag gtt gtg ctg gcg gag acc acc      816
Ile Ala Ala Ala Lys Met Gln Gly Lys Val Val Leu Ala Glu Thr Thr
            260                 265                 270

act gca cat gcc acg ctg aca ggc tta cac tac tac cac cag gac tgg      864
Thr Ala His Ala Thr Leu Thr Gly Leu His Tyr Tyr His Gln Asp Trp
        275                 280                 285

tcc cac gcg gct gcc tat gtc acg gtg cct ccc ctg aga ctg gac acc      912
Ser His Ala Ala Ala Tyr Val Thr Val Pro Pro Leu Arg Leu Asp Thr
    290                 295                 300

aac acc tca acc tac ctc atg agc ctg ctg gcc aat gac act ctg aac      960
Asn Thr Ser Thr Tyr Leu Met Ser Leu Leu Ala Asn Asp Thr Leu Asn
305                 310                 315                 320

atc gtg gca tca gat cac cgg cct ttc acc aca aag cag aaa gct atg     1008
Ile Val Ala Ser Asp His Arg Pro Phe Thr Thr Lys Gln Lys Ala Met
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ggc aag gaa gac ttc acc aag atc cca cat gga gtg agt ggc gtg cag      1056
Gly Lys Glu Asp Phe Thr Lys Ile Pro His Gly Val Ser Gly Val Gln
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gac cgc atg agc gtc atc tgg gag aga gga gtg gtt gga gga aag atg      1104
Asp Arg Met Ser Val Ile Trp Glu Arg Gly Val Val Gly Gly Lys Met
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gat gag aac cgt ttt gtg gcc gtt acc agt tcc aac gca gct aag ctt      1152
Asp Glu Asn Arg Phe Val Ala Val Thr Ser Ser Asn Ala Ala Lys Leu
    370                 375                 380

ctg aac ctg tat ccc cgc aag ggc cgc att att ccc gga gcc gat gct      1200
Leu Asn Leu Tyr Pro Arg Lys Gly Arg Ile Ile Pro Gly Ala Asp Ala
385                 390                 395                 400

gat gtg gtg gtg tgg gac cca gaa gcc aca aag acc atc tca gcc agc      1248
Asp Val Val Val Trp Asp Pro Glu Ala Thr Lys Thr Ile Ser Ala Ser
                405                 410                 415

acg cag gtc cag gga gga gac ttc aac ctg tat gag aac atg cgc tgc      1296
Thr Gln Val Gln Gly Gly Asp Phe Asn Leu Tyr Glu Asn Met Arg Cys
            420                 425                 430

cac ggc gtg cca ctg gtc acc atc agc cgg ggg cgc gtc gtg tat gag      1344
His Gly Val Pro Leu Val Thr Ile Ser Arg Gly Arg Val Val Tyr Glu
        435                 440                 445

aac ggc gtc ttc atg tgc gcc gag ggc acc ggc aag ttc tgt ccc ctg      1392
Asn Gly Val Phe Met Cys Ala Glu Gly Thr Gly Lys Phe Cys Pro Leu
    450                 455                 460

agg tcc ttc cca gac act gtc tac aag aag ctg gtc cag aga gag aag      1440
Arg Ser Phe Pro Asp Thr Val Tyr Lys Lys Leu Val Gln Arg Glu Lys
465                 470                 475                 480

act tta aag gtt aga gga gtg gac cgc act ccc tac ctg ggg gat gtc      1488
Thr Leu Lys Val Arg Gly Val Asp Arg Thr Pro Tyr Leu Gly Asp Val
                485                 490                 495

gct gtt gtc gtg cac cct ggg aaa aaa gag atg gga acc cca ctc gca      1536
Ala Val Val Val His Pro Gly Lys Lys Glu Met Gly Thr Pro Leu Ala
            500                 505                 510

gac act cct acc cgg ccc gtc acc cgg cat ggg ggc atg agg gac ctt      1584
Asp Thr Pro Thr Arg Pro Val Thr Arg His Gly Gly Met Arg Asp Leu
        515                 520                 525

cac gaa tcc agc ttc agc ctc tct ggc tct cag atc gat gac cat gtt      1632
His Glu Ser Ser Phe Ser Leu Ser Gly Ser Gln Ile Asp Asp His Val
    530                 535                 540

cca aag cga gct tca gct cgg atc ctc gct cct ccc gga ggc agg tcg      1680
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| Val | Val | Asn | Asp | Asp | Cys | Thr | His | Glu | Ala | Asp | Val | Tyr | Ile | Glu | Asn |
| | | | 20 | | | | | 25 | | | | | 30 | | |
| Gly | Ile | Ile | Gln | Gln | Val | Gly | Arg | Glu | Leu | Met | Ile | Pro | Gly | Gly | Ala |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Lys | Val | Ile | Asp | Ala | Thr | Gly | Lys | Leu | Val | Ile | Pro | Gly | Gly | Ile | Asp |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Thr | Ser | Thr | His | Phe | His | Gln | Thr | Phe | Met | Asn | Ala | Thr | Cys | Val | Asp |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Asp | Phe | Tyr | His | Gly | Thr | Lys | Ala | Ala | Leu | Val | Gly | Gly | Thr | Thr | Met |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Ile | Ile | Gly | His | Val | Leu | Pro | Asp | Lys | Glu | Thr | Ser | Leu | Val | Asp | Ala |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Tyr | Glu | Lys | Cys | Arg | Gly | Leu | Ala | Asp | Pro | Lys | Val | Cys | Cys | Asp | Tyr |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Ala | Leu | His | Val | Gly | Ile | Thr | Trp | Trp | Ala | Pro | Lys | Val | Lys | Ala | Glu |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Met | Glu | Thr | Leu | Val | Arg | Glu | Lys | Gly | Val | Asn | Ser | Phe | Gln | Met | Phe |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Met | Thr | Tyr | Lys | Asp | Leu | Tyr | Met | Leu | Arg | Asp | Ser | Glu | Leu | Tyr | Gln |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Val | Leu | His | Ala | Cys | Lys | Asp | Ile | Gly | Ala | Ile | Ala | Arg | Val | His | Ala |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Glu | Asn | Gly | Glu | Leu | Val | Ala | Glu | Gly | Ala | Lys | Glu | Ala | Leu | Asp | Leu |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Gly | Ile | Thr | Gly | Pro | Glu | Gly | Ile | Glu | Ile | Ser | Arg | Pro | Glu | Glu | Leu |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Glu | Ala | Glu | Ala | Thr | His | Arg | Val | Ile | Thr | Ile | Ala | Asn | Arg | Thr | His |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Cys | Pro | Ile | Tyr | Leu | Val | Asn | Val | Ser | Ser | Ile | Ser | Ala | Gly | Asp | Val |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Ile | Ala | Ala | Ala | Lys | Met | Gln | Gly | Lys | Val | Val | Leu | Ala | Glu | Thr | Thr |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Thr | Ala | His | Ala | Thr | Leu | Thr | Gly | Leu | His | Tyr | Tyr | His | Gln | Asp | Trp |
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| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Ile | Val | Ala | Ser | Asp | His | Arg | Pro | Phe | Thr | Thr | Lys | Gln | Lys | Ala | Met |
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| | | 355 | | | | | 360 | | | | | 365 | | | |
| Asp | Glu | Asn | Arg | Phe | Val | Ala | Val | Thr | Ser | Ser | Asn | Ala | Ala | Lys | Leu |

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    370                 375                 380
Leu Asn Leu Tyr Pro Arg Lys Gly Arg Ile Ile Pro Gly Ala Asp Ala
385                 390                 395                 400
Asp Val Val Val Trp Asp Pro Glu Ala Thr Lys Thr Ile Ser Ala Ser
                405                 410                 415
Thr Gln Val Gln Gly Gly Asp Phe Asn Leu Tyr Glu Asn Met Arg Cys
            420                 425                 430
His Gly Val Pro Leu Val Thr Ile Ser Arg Gly Arg Val Val Tyr Glu
        435                 440                 445
Asn Gly Val Phe Met Cys Ala Glu Gly Thr Gly Lys Phe Cys Pro Leu
    450                 455                 460
Arg Ser Phe Pro Asp Thr Val Tyr Lys Lys Leu Val Gln Arg Glu Lys
465                 470                 475                 480
Thr Leu Lys Val Arg Gly Val Asp Arg Thr Pro Tyr Leu Gly Asp Val
                485                 490                 495
Ala Val Val Val His Pro Gly Lys Lys Glu Met Gly Thr Pro Leu Ala
            500                 505                 510
Asp Thr Pro Thr Arg Pro Val Thr Arg His Gly Gly Met Arg Asp Leu
        515                 520                 525
His Glu Ser Ser Phe Ser Leu Ser Gly Ser Gln Ile Asp Asp His Val
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Pro Lys Arg Ala Ser Ala Arg Ile Leu Ala Pro Pro Gly Gly Arg Ser
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Ser Gly Ile Trp
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